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Figure 1   **Sequence alignment of mouse Serca 1, 2 and 3 protein.**

```
5   Serca1a    1 MEAAHSKSTEECLSYFGVSETTGLTPDQVKRHLEKYGPNELPAEEGKSLWELVVEQFEDL
    Serca2a    1 ..N..T.TV..V.GH...N.'S...SLE...KLK.RW.S.........T.L...I......
    Serca2b    1 ..N..T.TV..V.GH...N.S...SLE...KLK.RW.S.........T.L...I......
    Serca3a    1 ..E..LL.AADV.RR.S.TAEG..SLE..TDAR.R.......T.................
    Serca3b    1 ..E..LL.AADV.RR.S.TAEG..SLE..TDAR.R.......T.................
10  Serca3c    1 ..E..LL.AADV.RR.S.TAEG..SLE..TDAR.R.......T.................

    Serca1a   61 LVRILLLAACISFVLAWFEEGEETVTAFVEPFVILLILIANAIVGVWQERNAENAIEALK
    Serca2a   61 ...........................I...............V.................
    Serca2b   61 ...........................I...............V.................
15  Serca3a   61 .........LV.............T......L..M...V.............S......
    Serca3b   61 .........LV.............T......L..M...V.............S......
    Serca3c   61 .........LV.............T.....L..M...V.............S......

    Serca1a  121 EYEPEMGKVYRADRKSVQRIKARDIVPGDIVEVAVGDKVPADIRILSIKSTTLRVDQSIL
20  Serca2a  121 ...........Q...........K.........I...........LT..............
    Serca2b  121 ...........Q...........K.........I...........LT..............
    Serca3a  121 .........I.S...G....R.....................L.LIE.............
    Serca3b  121 .........I.S...G....R.....................L.LIE.............
    Serca3c  121 .........I.S...G....R.....................L.LIE.............
25
    Serca1a  181 TGESVSVIKHTDPVPDPRAVNQDKKNMLFSGTNIAAGKAVGIVATTGVSTEIGKIRDQMA
    Serca2a  181 .................................M.V.VA...N........E.V
    Serca2b  181 .................................M.V.VA...N........E.V
    Serca3a  181 .......T....AI...................S...L.VAVA..LQ..L....S...
30  Serca3b  181 .......T....AI...................S...L.VAVA..LQ..L....S...
    Serca3c  181 .......T....AI...................S..L.VAVA..LQ..L....S...

    Serca1a  241 ATEQDKTPLQQKLDEFGEQLSKVISLICVAVWLINIGHFNDPVHGGSWFRGAIYYFKIAV
    Serca2a  241 ....ER...................I...I................I............
35  Serca2b  241 ....ER...................I...I................I............
    Serca3a  241 .V.PER....R......R...HA..V......V......A..A.....L...V.......
    Serca3b  241 .V.PER....R......R...HA..V......V......A..A.....L...V.......
    Serca3c  241 .V.PER....R......R...HA..V......V......A..A.....L...V.......

40  Serca1a  301 ALAVAAIPEGLPAVITTCLALGTRRMAKKNAIVRSLPSVETLGCTSVICSDKTGTLTTNQ
    Serca2a  301 ............................................................
    Serca2b  301 ............................................................
    Serca3a  301 ......................R.....................................
    Serca3b  301 ......................R.....................................
45  Serca3c  301 ......................R.....................................

    Serca1a  361 MSVCKMFIIDKVDGDVCSLNEFSITGSTYAPEGEVLKNDKPVRAGQYDGLVELATICALC
    Serca2a  361 ....R...L...E..T...............I...Q.D....KCH...............
    Serca2b  361 ....R...L...E..T...............I...Q.D....KCH...............
50  Serca3a  361 ....R..VVAEAEAGT.R.H..T.S.T..T....RQGEQ...C..F.............
    Serca3b  361 ....R..VVAEAEAGT.R.H..T.S.T..T....RQGEQ...C..F.............
    Serca3c  361 ....R..VVAEAEAGT.R.H..T.S.T..T....RQGEQ...C..F.............

    Serca1a  421 NDSSLDFNETKGVYEKVGEATETALTTLVEKMNVFNTEVRSLSKVERANACNSVIRQLMK
55  Serca2a  421 ...A..Y..A.................C........D..LKG...I..........K....
    Serca2b  421 ...A..Y..A.................C........D..LKG...I..........K....
    Serca3a  421 ...A..Y..A.................C........D.DLKG..R....G......K...R
    Serca3b  421 ...A..Y..A.................C........D.DLKG..R....G......K...R
    Serca3c  421 ...A..Y..A.................C........D.DLKG..R....G......K...R
```

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```
      Serca1a  481  KEFTLEFSRDRKSMSVYCSPAKSSRAAVGNKMFVKGAPEGVIDRCNYVRVGTTRVPLTGP
      Serca2a  481  ................T.N.P..TSMS-.............THI...S.K..M.PG
      Serca2b  481  ................T.N.P..TSMS-.............THI...S.K..M.PG
      Serca3a  481  ................T.TRADPKVQ.S.........S..E..SS....SRTA..STT
  5   Serca3b  481  ................T.TRADPKVQ.S.........S..E..SS....SRTA..STT
      Serca3c  481  ................T.TRADPKVQ.S.........S..E..SS....SRTA..STT

      Serca1a  541  VKEKIMSVIKEWGTGRDTLRCLALATRDTPPKREEMVLDDSAKFMEYEMDLTFVGVVGML
      Serca2a  540  ..Q......R...S.S...........H.N.L.....H.E...N.IK..TN.....C....
 10   Serca2b  540  ..Q......R...S.S...........H.N.L.....H.E...N.IK..TN.....C....
      Serca3a  541  SR.H.LAK.RD..S.S...............RK.D.H...CSR.VQ..T......C....
      Serca3b  541  SR.H.LAK.RD..S.S...............RK.D.H...CSR.VQ..T......C....
      Serca3c  541  SR.H.LAK.RD..S.S...............RK.D.H...CSR.VQ..T......C....

 15   Serca1a  601  DPPRKEVTGSIQLCRDAGIRVIMITGDNKGTAIAICRRIGIFSENEEVTDRAYTGREFDD
      Serca2a  600  ....I..AS.VK...Q................V.........GQD.D..SK.F......E
      Serca2b  600  ....I..AS.VK...Q................V.........GQD.D..SK.F......E
      Serca3a  601  ....P..AAC.TR.SR.....V..........V.....L...GDT.D.LGK........
      Serca3b  601  ....P..AAC.TR.SR.....V..........V.....L...GDT.D.LGK........
 20   Serca3c  601  ....P..AAC.TR.SR.....V..........V.....L...GDT.D.LGK........

      Serca1a  661  LPLAEQREACRRACCFARVEPSHKSKIVEYLQSYDEITAMTGDGVNDAPALKKAEIGIAM
      Serca2a  660  .SPSA..D..LN.R...............F...F..................S.....
      Serca2b  660  .SPSA..D..LN.R...............F...F..................S.....
 25   Serca3a  661  .SPEQ..Q...T.R.......A...R...N...FN.......................
      Serca3b  661  .SPEQ..Q...T.R.......A...R...N...FN.......................
      Serca3c  661  .SPEQ..Q...T.R.......A...R...N...FN.......................

      Serca1a  721  GSGTAVAKTASEMVLADDNFSTIVAAVEEGRAIYNNMKQFIRYLISSNVGEVVCIFLTAA
 30   Serca2a  720  ...........................................................
      Serca2b  720  ...........................................................
      Serca3a  721  ........S.A....S....AS.....................................I
      Serca3b  721  ........S.A....S....AS.....................................I
      Serca3c  721  ........S.A....S....AS.....................................I
 35
      Serca1a  781  LGLPEALIPVQLLWVNLVTDGLPATALGFNPPDLDIMDRPPRSPKEPLISGWLFFRYMAI
      Serca2a  780  ..F...........................NK...N.............L..
      Serca2b  780  ..F...........................NK...N.............L..
      Serca3a  781  ..........................EK...N.R.A..........L..
 40   Serca3b  781  ..........................EK...N.R.A..........L..
      Serca3c  781  ..........................EK...N.R.A..........L..

      Serca1a  841  GGYVGAATVGAAAWWFLYAEDGPHVSYHQLTHFMQCTEHNPEFDGLDCEVFEAPEPMTMA
      Serca2a  840  .C..............IA.DG..R..FY..S..L..K.D..D...V..AI..S.Y.....
 45   Serca2b  840  .C..............IA.DG..R..FY..S..L..K.D..D...V..AI..S.Y.....
      Serca3a  841  .V...L...A..T.....DAE..Q.TFY..RN.LK.S.D..L.A.I..K...SRF.T...
      Serca3b  841  .V...L...A..T.....DAE..Q.TFY..RN.LK.S.D..L.A.I..K...SRF.T...
      Serca3c  841  .V...L...A..T.....DAE..Q.TFY..RN.LK.S.D..L.A.I..K...SRF.T...

 50   Serca1a  901  LSVLVTIEMCNALNSLSENQSLLRMPPWVNIWLLGSICLSMSLHFLILYVDPLPMIFKLR
      Serca2a  900  .......................E....V...............E...L..QIT
      Serca2b  900  .......................E....V...............E...L..QIT
      Serca3a  901  ..............V..........L.P....AVVM..A......L.P...L..QVT
      Serca3b  901  ..............V..........L.P....AVVM..A......L.P...L..QVT
 55   Serca3c  901  ..............V..........L.P....AVVM..A......L.P...L..QVT
```

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```
      Sercala    961  ALDFTQWLMVLKISLPVIGLDELLKFIARNYLEG
      Serca2a    960  P.NL..............LM..T...V......QPAILE
      Serca2b    960  P.NL..............LM..T...V......QPGKECVQPATKSSCSLSACTDGISWP
      Serca3a    961  P.SGR..GV..QM.....L...A..YLS..HMDEKKDLK
 5    Serca3b    961  P.SGR..GV..QM.....L...A..YLS..HMD.VLGTFMQARSRQLPTTSRTPYHTGKK
      Serca3c    961  P.SGR..GV..QM.....L...A..YLS..HMD.VLGTFMQARSRQLPTTSRTPYHTGLA


      Serca2b   1020  FVLLIMPLVVWVYSTDTNFSDMFWS
10    Serca3b   1021  GPEVNPGSRGESPVWPSD
      Serca3c   1021  SWKKRT



15
```

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Figure 2　　　　　　**Sequence similarity of Serca2 proteins in mammalian species**

```
      Mouse_2a      1 MENAHTKTVEEVLGHFGVNESTGLSLEQVKKLKERWGSNELPAEEGKTLLELVIEQFEDL
  5   Mouse_2b      1 ...........................................................
      Rat_2b        1 ...........................................................
      Rat_2a        1 ...........................................................
      Dog_2a        1 ...........................................................
      Cat_2a        1 ............Y..............................................
 10   Pig_2a        1 ...........................................................
      Pig_2b        1 ...........................................................
      Human_2b      1 ...........................................................
      Human_2c      1 ...........................................................
      Human_2a      1 ...........................................................
 15   Rabbit_2a     1 ...........................................................
      Rabbit_2b     1 ...........................................................

      Mouse_2a     61 LVRILLLAACISFVLAWFEEGEETITAFVEPFVILLILVANAIVGVWQERNAENAIEALK
      Mouse_2b     61 ...........................................................
 20   Rat_2b       61 ...........................................................
      Rat_2a       61 ...........................................................
      Dog_2a       61 ...........................................................
      Cat_2a       61 ...........................................................
      Pig_2a       61 ...........................................................
 25   Pig_2b       61 ...........................................................
      Human_2b     61 ...........................................................
      Human_2c     61 ...........................................................
      Human_2a     61 ...........................................................
      Rabbit_2a    61 ...........................................................
 30   Rabbit_2b    61 ...........................................................

      Mouse_2a    121 EYEPEMGKVYRQDRKSVQRIKAKDIVPGDIVEIAVGDKVPADIRLTSIKSTTLRVDQSIL
      Mouse_2b    121 ...........................................................
      Rat_2b      121 ...........................................................
 35   Rat_2a      121 ...........................................................
      Dog_2a      121 ...........................................................
      Cat_2a      121 ...........................................................
      Pig_2a      121 ...........................................................
      Pig_2b      121 ...........................................................
 40   Human_2b    121 ...........................................................
      Human_2c    121 ...........................................................
      Human_2a    121 ...........................................................
      Rabbit_2a   121 ...........................................................
      Rabbit_2b   121 ...........................................................
 45
      Mouse_2a    181 TGESVSVIKHTDPVPDPRAVNQDKKNMLFSGTNIAAGKAMGVVVATGVNTEIGKIRDEMV
      Mouse_2b    181 ...........................................................
      Rat_2b      181 ...........................................................
      Rat_2a      181 ...........................................................
 50   Dog_2a      181 ...........................................................
      Cat_2a      181 ...........................................................
      Pig_2a      181 ...........................................................
      Pig_2b      181 ...........................................................
      Human_2b    181 ...........................................................
 55   Human_2c    181 ...........................................................
      Human_2a    181 ...........................................................
      Rabbit_2a   181 ...........................................................
      Rabbit_2b   181 ...........................................................
```

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```
       Mouse_2a   241 ATEQERTPLQQKLDEFGEQLSKVISLICIAVWIINIGHFNDPVHGGSWIRGAIYYFKIAV
       Mouse_2b   241 ............................................................
       Rat_2b     241 ............................................................
   5   Rat_2a     241 ............................................................
       Dog_2a     241 ............................................................
       Cat_2a     241 ............................................................
       Pig_2a     241 ............................................................
       Pig_2b     241 ............................................................
  10   Human_2b   241 ............................................................
       Human_2c   241 ............................................................
       Human_2a   241 ............................................................
       Rabbit_2a  241 ............................................................
       Rabbit_2b  241 ............................................................
  15
       Mouse_2a   301 ALAVAAIPEGLPAVITTCLALGTRRMAKKNAIVRSLPSVETLGCTSVICSDKTGTLTTNQ
       Mouse_2b   301 ............................................................
       Rat_2b     301 ............................................................
       Rat_2a     301 ............................................................
  20   Dog_2a     301 ............................................................
       Cat_2a     301 ............................................................
       Pig_2a     301 ............................................................
       Pig_2b     301 ............................................................
       Human_2b   301 ............................................................
  25   Human_2c   301 ............................................................
       Human_2a   301 ............................................................
       Rabbit_2a  301 ............................................................
       Rabbit_2b  301 ............................................................

  30   Mouse_2a   361 MSVCRMFILDKVEGDTCSLNEFSITGSTYAPIGEVQKDDKPVKCHQYDGLVELATICALC
       Mouse_2b   361 ............................................................
       Rat_2b     361 ........................T...................................
       Rat_2a     361 ........................T...................................
       Dog_2a     361 ..........R....S........T..................H................
  35   Cat_2a     361 ..........R....S........T..................H................
       Pig_2a     361 ........................T..................H................
       Pig_2b     361 ........................T..................H................
       Human_2b   361 ..........R.............T..................H......N.........
       Human_2c   361 ..........R.............T..................H......N.........
  40   Human_2a   361 ..........R.............T..................H......N.........
       Rabbit_2a  361 ..............D.........T..................H................
       Rabbit_2b  361 ..............D.........T..................H................

       Mouse_2a   421 NDSALDYNEAKGVYEKVGEATETALTCLVEKMNVFDTELKGLSKIERANACNSVIKQLMK
  45   Mouse_2b   421 ............................................................
       Rat_2b     421 ............................................................
       Rat_2a     421 ............................................................
       Dog_2a     421 ............................................................
       Cat_2a     421 ..............K.F...........................................
  50   Pig_2a     421 ............................................................
       Pig_2b     421 ............................................................
       Human_2b   421 ............................................................
       Human_2c   421 ............................................................
       Human_2a   421 ............................................................
  55   Rabbit_2a  421 ............................................................
       Rabbit_2b  421 ............................................................
```

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```
     Mouse_2a   481  KEFTLEFSRDRKSMSVYCTPNKPSRTSMSKMFVKGAPEGVIDRCTHIRVGSTKVPMTPGV
     Mouse_2b   481  ............................................................
     Rat_2b     481  ............................................................
     Rat_2a     481  ............................................................
 5   Dog_2a     481  ............................................................
     Cat_2a     481  ............................................................
     Pig_2a     481  ............................................................
     Pig_2b     481  ............................................................
     Human_2b   481  .........................................................S..
10   Human_2c   481  .........................................................S..
     Human_2a   481  .........................................................S..
     Rabbit_2a  481  .........................................................A..
     Rabbit_2b  481  .........................................................A..

15   Mouse_2a   541  KQKIMSVIREWGSGSDTLRCLALATHDNPLKREEMHLEDSANFIKYETNLTFVGCVGMLD
     Mouse_2b   541  ............................................................
     Rat_2b     541  ............................R...............................
     Rat_2a     541  ............................R...............................
     Dog_2a     541  ...V........................R.....N.........................
20   Cat_2a     541  ...V........................R.....N.........................
     Pig_2a     541  ...........................MR.....N.........................
     Pig_2b     541  ...........................MR.....N.........................
     Human_2b   541  ............................R...............................
     Human_2c   541  ............................R...............................
25   Human_2a   541  ............................R...............................
     Rabbit_2a  541  ............................R.........K.....................
     Rabbit_2b  541  ............................R.........K.....................

     Mouse_2a   601  PPRIEVASSVKLCRQAGIRVIMITGDNKGTAVAICRRIGIFGQDEDVTSKAFTGREFDEL
30   Mouse_2b   601  ............................................................
     Rat_2b     601  ............................................................
     Rat_2a     601  ............................................................
     Dog_2a     601  ............................................................
     Cat_2a     601  ............................................................
35   Pig_2a     601  ............................................................
     Pig_2b     601  ............................................................
     Human_2b   601  ............................................................
     Human_2c   601  ............................................................
     Human_2a   601  ............................................................
40   Rabbit_2a  601  ..............................................E....A........
     Rabbit_2b  601  ..............................................E....A........

     Mouse_2a   661  SPSAQRDACLNARCFARVEPSHKSKIVEFLQSFDEITAMTGDGVNDAPALKKSEIGIAMG
     Mouse_2b   661  ............................................................
45   Rat_2b     661  ............................................................
     Rat_2a     661  ............................................................
     Dog_2a     661  ............................................................
     Cat_2a     661  ............................................................
     Pig_2a     661  N.....E.....................................................
50   Pig_2b     661  N.....E.....................................................
     Human_2b   661  N..................................................A.......
     Human_2c   661  N..................................................A.......
     Human_2a   661  N..................................................A.......
     Rabbit_2a  661  N..................................................A.......
55   Rabbit_2b  661  N..................................................A.......
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```
     Mouse_2a   721 SGTAVAKTASEMVLADDNFSTIVAAVEEGRAIYNNMKQFIRYLISSNVGEVVCIFLTAAL
     Mouse_2b   721 ...........................................................
     Rat_2b     721 ...........................................................
     Rat_2a     721 ...........................................................
   5 Dog_2a     721 ...........................................................
     Cat_2a     721 ...........................................................
     Pig_2a     721 ...........................................................
     Pig_2b     721 ...........................................................
     Human_2b   721 ...........................................................
  10 Human_2c   721 ...........................................................
     Human_2a   721 ...........................................................
     Rabbit_2a  721 ...........................................................
     Rabbit_2b  721 ...........................................................

  15 Mouse_2a   781 GFPEALIPVQLLWVNLVTDGLPATALGFNPPDLDIMNKPPRNPKEPLISGWLFFRYLAIG
     Mouse_2b   781 ...........................................................
     Rat_2b     781 ...........................................................
     Rat_2a     781 ...........................................................
     Dog_2a     781 ...........................................................
  20 Cat_2a     781 ...........................................................
     Pig_2a     781 ...........................................................
     Pig_2b     781 ...........................................................
     Human_2b   781 ...........................................................
     Human_2c   781 ...........................................................
  25 Human_2a   781 ...........................................................
     Rabbit_2a  781 ...........................................................
     Rabbit_2b  781 ...........................................................

     Mouse_2a   841 CYVGAATVGAAAWWFIAADGGPRVSFYQLSHFLQCKEDNPDFDGVDCAIFESPYPMTMAL
  30 Mouse_2b   841 ...........................................................
     Rat_2b     841 ...............................................E...........
     Rat_2a     841 ...............................................E...........
     Dog_2a     841 .........................................D.....E...........
     Cat_2a     841 .........................................D.....E...........
  35 Pig_2a     841 ...................................T...........E.....V.....
     Pig_2b     841 ...................................T...........E.....V.....
     Human_2b   841 ...............................................E...........
     Human_2c   841 ...............................................E...........
     Human_2a   841 ...............................................E...........
  40 Rabbit_2a  841 ...............................................E...........
     Rabbit_2b  841 ...............................................E...........

     Mouse_2a   901 SVLVTIEMCNALNSLSENQSLLRMPPWENIWLVGSICLSMSLHFLILYVEPLPLIFQITP
     Mouse_2b   901 ...........................................................
  45 Rat_2b     901 ...........................................................
     Rat_2a     901 ...........................................................
     Dog_2a     901 ...........................................................
     Cat_2a     901 ...........................................................
     Pig_2a     901 ...........................................................
  50 Pig_2b     901 ...........................................................
     Human_2b   901 ...........................................................
     Human_2c   901 ...........................................................
     Human_2a   901 ...........................................................
     Rabbit_2a  901 ...........................................................
  55 Rabbit_2b  901 ...........................................................
```

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```
      Mouse_2a    961  LNLTQWLMVLKISLPVILMDETLKFVARNYLEQPAILE-----------------------
      Mouse_2b    961  ..................................GKECVQPATKSSCSLSACTDGISWPF
      Rat_2b      961  ...............................-.GKECA.....P..............
      Rat_2a      961  ...............................-.AILE
  5   Dog_2a      961  ...............................-.AILE
      Cat_2a      961  ...............................-.AILE
      Pig_2a      961  ...............................-.AILE
      Pig_2b      961  ...............................-.GKEC........-..F..........
      Human_2b    961  ..V............................-.GKEC........-..F..........
 10   Human_2c    961  ..V............................-.VLSSL
      Human_2a    961  ..V............................-.AILE
      Rabbit_2a   961  ..V............................-.AILE
      Rabbit_2b   961  ..V............................-.GKEC....PQ.-...W...E.V....


 15   Mouse_2b   1021  VLLIMPLVVWVYSTDTNFSDMFWS
      Rat_2b     1020  ........................
      Pig_2b     1019  ........................
      Human_2b   1019  ........................
      Rabbit_2b  1019  ....V...M..........LL..
 20
```

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Fig. 3 Targeting construct for Serca2 flox gene modification. Sequence information.

———————— Serca2 gene ————————————

LoxP site 1: Intron 1

underlined sequence = loxP site and cloning sequence

Exon I (partial)

CACGGGGCTGAGCTTGGAGCAGGTCAAGAAGCTCAAGGAGAGATGGGGCTCCAACGgtaggtgcggggcg
ccggggctgcagcggcgcgggcgcgggcccgagcgccaaggaagatggctgacccggctccacctcgtggg
gcttggctcggcgcggcgcccgacggctgcgagaggccggcggtccacgcgcgggtctgggccatcgccg
accttagggggtctcgaatcaagcttatcgataccgtcgatcggacctcgagggggggcccggtacccggg
gatcaattcgagctcgcccggggatcgatccggaacccttaatATAACTTCGTATAATGTATGCTATACG
AAGTTATtaggtccctcgacctgcagcccaagctccGGGGAtctcgagccggtgaccttcccggccggcg
ctcagcgagtcggattgggggggggggagagggagtgggaaggagggaggaggttcctgcggctgggctg
agtcccccgcggatttatgaggcgtcgatgttgttgagaaaccctcggaccgtttcttgtgctccccaaa
gttgcacatctggcagaagtgatgacccagctgaaatgactgcatggtcctggaggccggagagggctta
cgggcagttccgaggccactgattaccagggctgaataattttctcggggtatcaaagtggagacagatt
gttgtacgttcatacacctatatccgccattcagacaacgatggtggtgaatttagcagtttttaataaa
agcgctaatacaatatcttcatttttctttc

———————————— Serca2 gene ————————————

LoxP site 2: Intron 3 5'of genomic XcmI site

underlined sequence = loxPsite, cloning sites and partial HSV-TK

ccaattttttattcttagaacattgtattcttatactgtgataggaagtgaaaaatcatacagtacttgtc
ttaggtttcacaaaactgataactgtatggtttcaattatgtattcacacgtttaagtctgacccagggG
GATCCggaacccttaatATAACTTCGTATAATGTATGCTATACGAAGTTATtaggtccctcgacctgcag
cccaagctgatcctctagtcgagccccagctggttctttccgcctcagaagccatagagcccaccgcatc
cccagcatgcctgctattgtcttcccaatcctcccccttgctgtcctgccccacccacccccagaata
gaatgacacctactcagacaatgcgatgcaattcctcattttattaggaaaggacagtgggagtggcac
cttccagggtcaaggaaggcacggggggagggcaaacaacagatggctggcaactagaaggcacagtcga
ggctgatcagcgagctctagctagagaattgatcccctcagaagaactcgtcaagaaggcgatagaaggc
gatgcgctgcgaatcgggagcggcgat*ccgtaaagcacgaggaagcgg*cagcccattcgccgccaagc
tctttcagcaatatcacgggtagccaacgctatgt*ctgataagcggtccgcccacacccaa*ccggcca
caagtc*atgaaatcca*aaaaagcgggccatttttccacc*atgatttt*cggcaagcaaggccttt*c
cattgggtcaccgac*aga*catt*tccgt*c*ggcattgcgc*ccct

———————————— HSV-TK Neo antibiotics cassette ————————————

LoxP site 3: Intron 3 5'of genomic XcmI site

underlined sequence = loxP site, cloning sites and partial Neo gene

gttttcat*accaccgcgggtcccgggc*gatat*ttcaccttgtc*ag*cggtgttgtgtggtgtaaatg
ttcgcgattgtttcgaaagcccc*agcacccgccagtaagtcatcggctcgggtacgtagacgatatcgtc
gcgcgaacccagggccaccagcaagttgcgtggtggtggttttccccatcc*gtggggac*gtctatataa
acc*gcagtagcgtgggcatttttctgctccgggcggacttccgtggcttcttgctgccggcgagggcgcaa
cgccgtacgtcggttgctatggccgcgagaacgcgcagcctggtcgaacgcagacgcgtgttgatggccgg
ggtacgaagccatacgcgcttctacaaggcgctggccgaagaggtgcgggagtttcacgccaccaagatct
gcggcacgctgttgacgctgttaagcgggtcgctgcagggtcgctcggtgttcgaggccacacgcgtcacc
ttaatatgcgaagtggacctcggaccgcgccgccccgactgcatctgcgtgttcgaattcgccaatgacaa
gacgctgggcggggtttgctcgacattgggtggaaacattccaggcctgggtggagaggctttttgcttcc
tcttgcaaaaccacactgctcgacattgggtggaaacattccaggcctgggtggagaggctttttgcttcc
tcttgaaaaccacactgctcgatccggaacccttaatATAACTTCGTATAATGTATGCTATACGAA
GTTATtaggtccctcgacctgcagcccaagctgatcctctagagtcgacctcga*tcgtggtcatggcctctatgaaa*
acattagcttagagg

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Fig. 4A **Schematic representation of genetic manipulation.**

Serca2 (*atp2a2*) gene modification

Exon #

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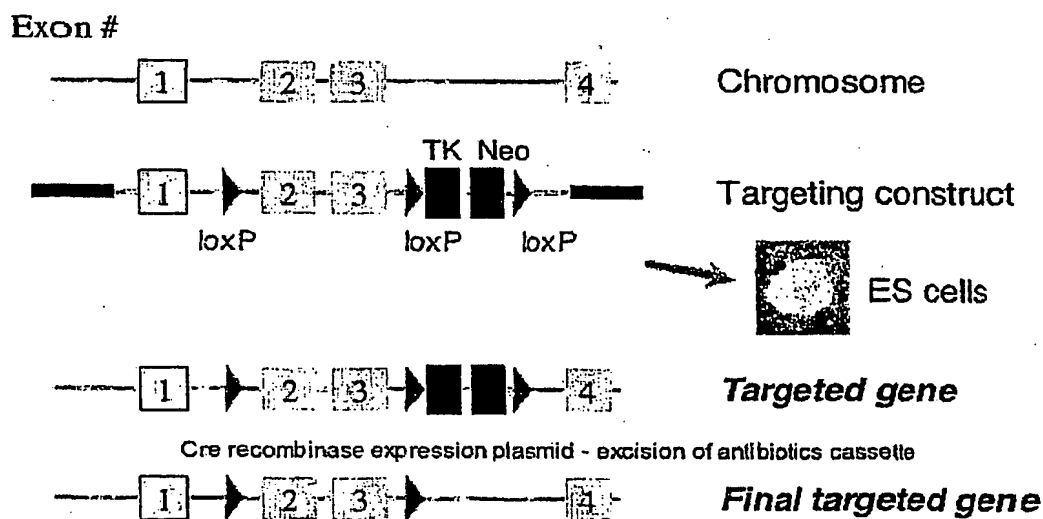
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Fig 4 B: Verification of Serca locus targeting events offspring from chimeric mice.

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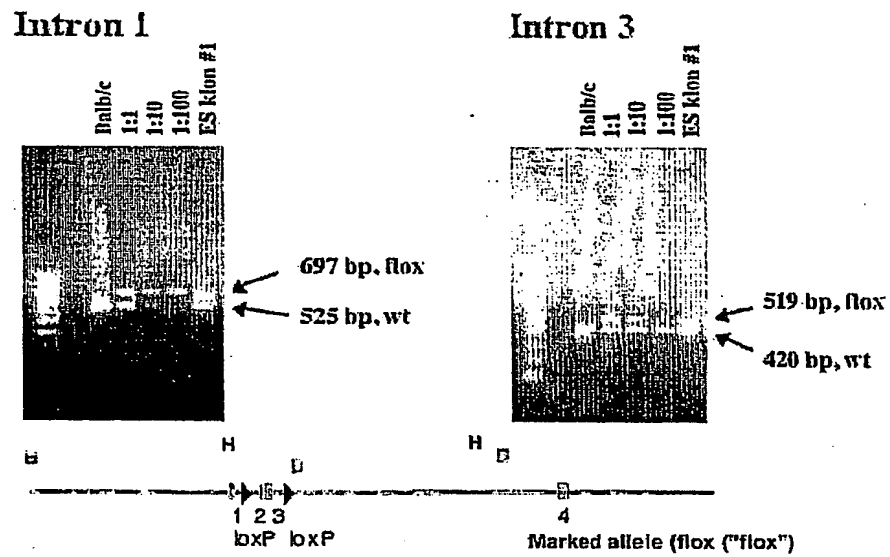
Fig. 6 Cardiac ANP mRNA expression.

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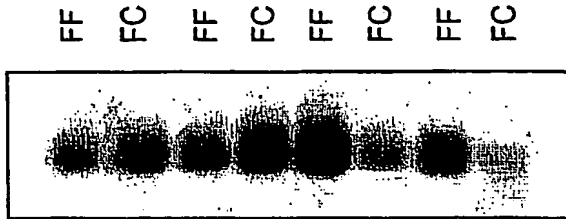
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Fig. 7 Serca2 protein expression.

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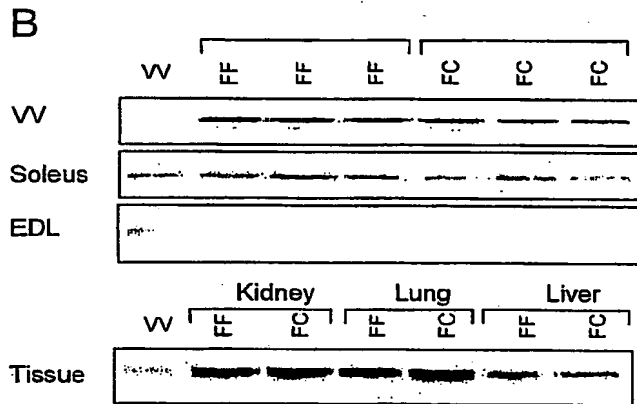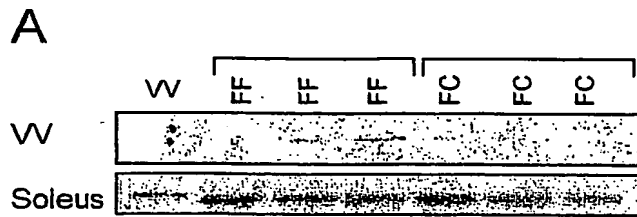
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Fig. 8 Compensatory mechanisms in Serca^{flox} MLC-2ν-Cre mice.

A

B

C

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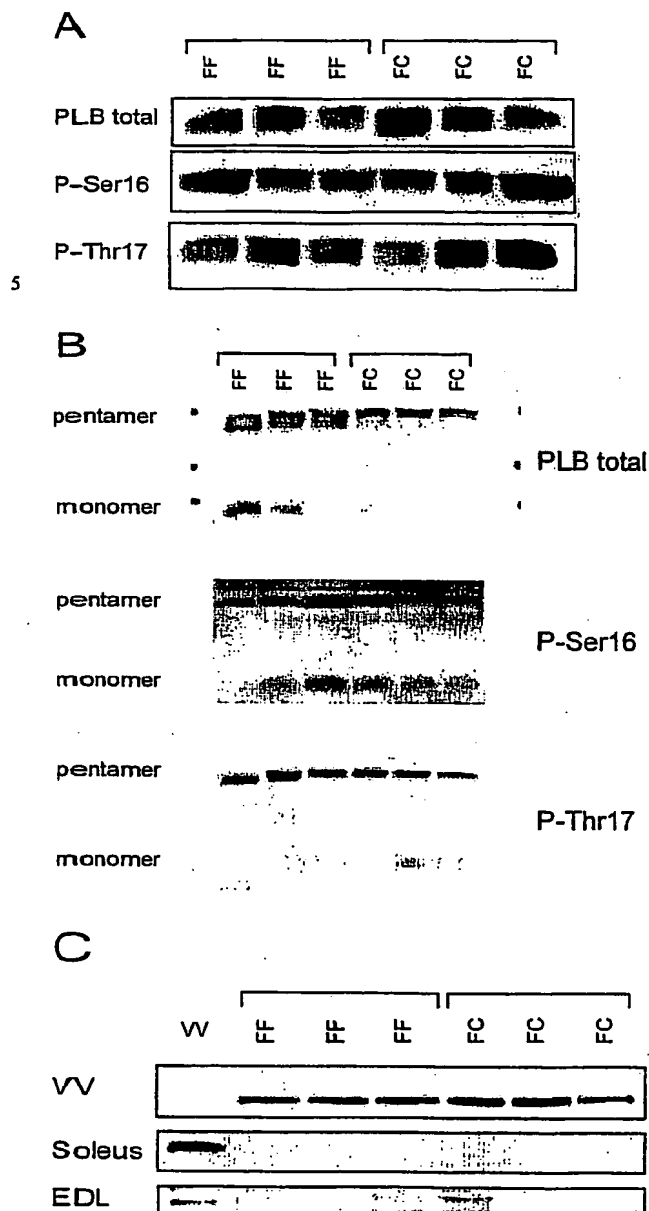
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Figure 9   **Genotypes PCR**

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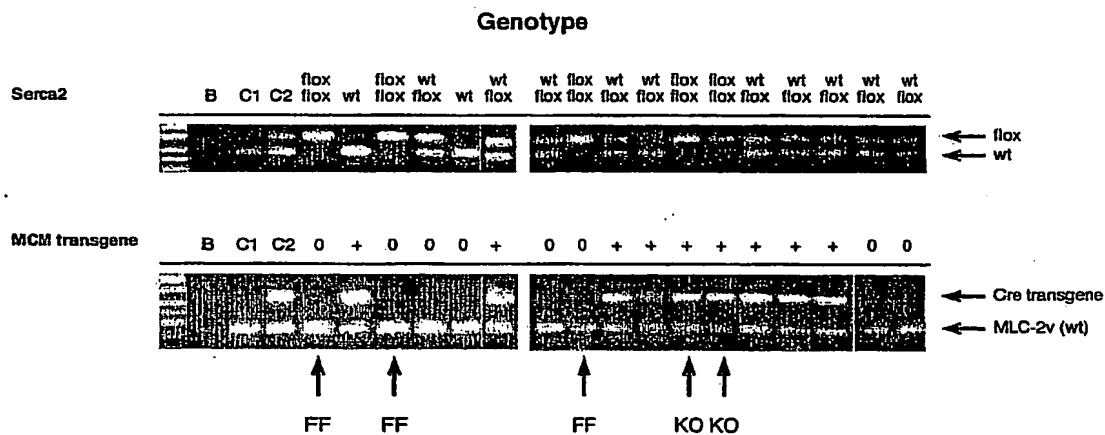
Generation of animals with Serca2^{flox} and MCM transgene alleles.

Genotypes FF. Serca2$^{flox/flox}$;   KO, Serca2$^{flox/flox}$ MCM

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Figure 10   Heart morphology

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Genotype

	FF	KO
43 d	8.3	8.1
52 d	7.4	7.3

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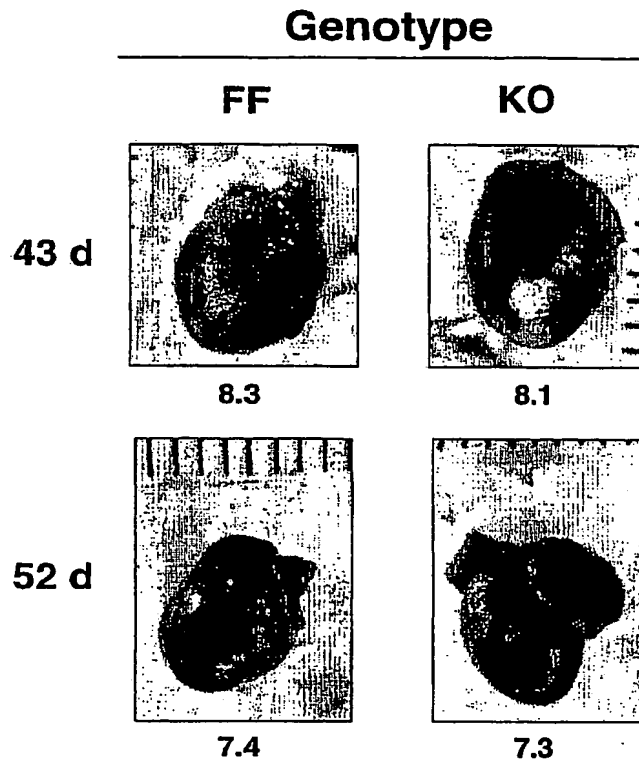
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Figure 11   Pilot series left atrial diastolic diameter.

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Tamoxifen-injected animals
43-52 days

10

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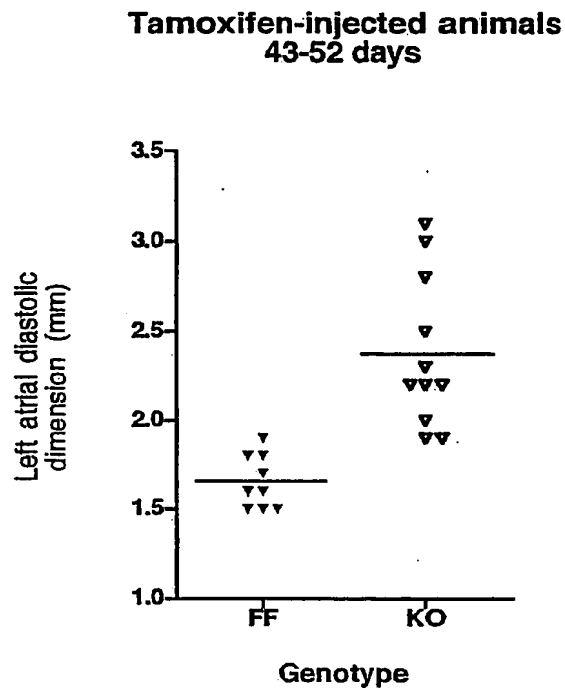
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Figure 12   Serca protein content in tamoxifen-induced FF and KO mice

A

B